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THE EFFECTS OF ACUTE STRESS AND OF ACTH UPON ASCORBIC ACID AND LIPID CONTENT OF THE ADRENAL GLANDS OF WILD RATS

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Most of our knowledge of the effects of stress upon adrenal cortical function is based upon the results of studies which made use of domesticated rats and guinea-pigs as experimental animals. When such domesticated mammals are exposed to stress, an initial metabolic depletion of the adrenal cortex appears to be the invariable result (Sayers, 1950). On the other hand, the limited amount of information available (Josephson, Taylor, Greenberg & Nadel, 1949; Jailer & Boas, 1950; Zarrow & Baldini, 1952) suggests that adrenal cortical ascorbic acid and lipid depletion may not occur when feral species are exposed to stress.

In these laboratories we have for several years been studying the wild strain of the Norway rat, domesticated strains of which species are commonly in use in laboratories throughout the world. The experiments to be reported here were designed to compare the effects of stress upon the adrenal cortex in both wild and domesticated strains of this species. Interest in the wild rat dates back at least 50 years, at which time the comparatively large adrenals of these animals were first reported (Watson, 1907). That wild rats do have adrenals approximately twice the size of those found in domesticated rats of equal body weight has been repeatedly confirmed (Hatai, 1914; King & Donaldson, 1929; Rogers & Richter, 1948; Woods, 1954), and it has been established that this difference in gland weight can be accounted for entirely by the amount of cortical tissue present (Donaldson, 1928; Rogers & Richter, 1948). So far, only a few attempts have been made to correlate this comparatively large amount of adrenal cortical tissue with the function of the organ (Rogers & Richter, 1948; Nichols, 1948; Richter, 1950; Covian, 1949). It has been suggested (Richter, 1950; Covian, 1949) that the large amount of adrenal cortical tissue present in the wild rat may confer upon this animal an increased capacity to withstand stress. On purely teleological grounds this suggestion appears to warrant consideration. The results reported here and others from long-term stress experiments (Woods, 1957) raise the question as to whether or not exposure to stress actually does result in increased adrenal cortical secretion in wild animals.

MATERIALS AND METHODS

Adult wild and domesticated Norway rats weighing between 150 and 500 g were used in these experiments. The wild rats were captured in the streets and alleys of Baltimore and were kept in the laboratory for at least 1 month before use. The domesticated rats were acquired from two sources. The majority came from the Richter colony; a few were from Carworth Farms stock.

All animals were housed in individual cages during an experiment. Usually they were so housed for several days before an experiment so that food and water intake could be recorded as a check on their general health.

In each experiment the rats were paired off with one wild and one domesticated rat in each pair. The pairs were then divided into experimental and control groups. Ordinarily, all of the experimental group in any one experiment were introduced into the experimental environment at once and then a pair of animals was removed for killing at appropriate times. In some cases (loud noise, fighting) this could not be done owing to limitations imposed by time or equipment.

Unless otherwise stated, all animals were killed instantaneously by shooting them through the chest with a 0·22 calibre bullet. Immediately after they were shot, they were tied to a dissecting board and their adrenals were removed within 2 min. The adrenals were dissected free of extraneous tissue under a dissecting microscope and weighed to within 0·1 mg on a torsion balance. One adrenal was preserved in buffered neutral formalin (10%); the other was macerated in 4% metaphosphoric acid for determination of ascorbic acid by a modification (Woods, 1954) of the method of Bessey & King (1933). The adrenals which were prepared in formalin were later halved for histological preparations. One half was embedded in paraffin, sectioned at 7 μ, and stained with haematoxylin and eosin; the other was frozen, sectioned at 15 μ, and stained with Sudan IV.

RESULTS

Exposure to cold. In a series of twelve experiments forty-one wild rats and forty-eight domesticated rats were exposed to low environmental temperatures for various periods of time up to 24 hr. For this purpose all the experimental animals were introduced, in their individual cages, into a walk-in type refrigerator ($6 \times 8 \times 6$ ft.). The temperature in this room varied between -2 and 4° C. In experiments lasting several hours the door was opened often enough to prevent freezing of drinking water. At intervals of 0·5, 1, 2, 3, 6 and 24 hr after the start of the exposure, a pair of rats was removed for autopsy. In addition, one experiment was carried out with wild rats only in which the exposure was for 12 hr.

The ascorbic acid contents of the adrenals of these rats are given in Table 1. As expected on the basis of previous work by others, the concentration of ascorbic acid in the adrenals of the domesticated rats was rapidly diminished during the first 2 hr of exposure to cold and control concentration was regained during subsequent hours of continuous exposure. This is in striking contrast to results obtained in the wild rat, in which no significant changes in adrenal ascorbic acid occurred during the same period under identical conditions. Microscopic examination of Sudan IV stained sections of the adrenals from these animals shows a comparable picture: whereas sudanophilic lipids were greatly reduced within 3 hr in the domesticated rats, no noticeable changes occurred in the adrenals of the wild rats.

TABLE 1. Effect of exposure to cold upon adrenal ascorbic acid in wild and domesticated rats

No. of rats	Exposure (hr)	Adrenal ascorbic acid (μg/mg)	P*
		Wild rats	
12	0	3·55 ± 0·23†	—
5	1	3·46 ± 0·41	>0·1
4	2	3·55 ± 0·45	>0·1
5	3	3·50 ± 0·33	>0·1
5	6	3·59 ± 0·41	>0·1
5	12	3·88 ± 0·32	>0·1
5	24	3·54 ± 0·46	>0·1
		Domesticated rats	
12	0	3·55 ± 0·32	—
6	1	2·06 ± 0·12	<0·001
6	2	2·36 ± 0·06	<0·01
6	3	2·48 ± 0·17	<0·01
6	6	2·68 ± 0·19	<0·02
12	24	3·26 ± 0·26	<0·1

* Each experimental group compared to control group.

† S.E. of the mean.

Auditory stimulation. In four experiments twenty-four pairs of rats were studied after exposure to a loud noise. The experimental animals were introduced into a partially sound-proof chamber (3·5 × 2·5 × 2·5 ft.). The stimulus was provided by a police whistle connected to a compressed air line which passed through a small hole in the wall of the chamber. At the beginning of the exposure period, pressure in the line was raised to 300 mm Hg and this pressure was maintained throughout the experiment. This arrangement produced an extremely loud, shrill noise. An estimate of the intensity of the stimulus was made by comparing it with the sound emitted by an audiometer set at 1024 c/s. On the basis of such a comparison, the stimulus was estimated to be 70–80 db above the threshold of the experimenter. In each experiment a pair of animals was removed from the sound chamber after 15, 30, 60, 90 and 120 min of stimulation. The controls were all killed immediately before the beginning of the stimulation.

The ascorbic acid contents of the adrenals of these animals are shown in Fig. 1. The results are consistent with those from the experiments described above in that (1) a marked depletion of ascorbic acid occurred in the adrenals of the domesticated rats, and (2) no changes were detected in the wild rats.

Surgical operations. Eighteen wild rats and six domesticated rats were subjected to surgical procedures under ether anaesthesia and adrenal ascorbic acid was determined 2 hr later. Two experiments were carried out. In the first six wild rats and six domesticated rats were unilaterally adrenalectomized through a dorsal approach. The wounds were closed with continuous sutures and the animals were returned to their cages. Two hours later they were again anaesthetized with ether and the remaining adrenal was removed for analysis.

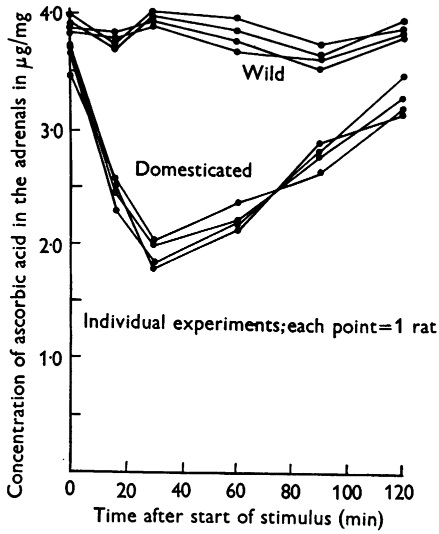

Fig. 1. To show the effects upon adrenal ascorbic acid of continuous auditory stimulation in wild and domesticated rats.

Such a surgical procedure with the attending anaesthesia produced a sharp decrease (55 %) in assayable ascorbic acid in the adrenals of the domesticated rats. The wild rats, in so far as adrenal ascorbic acid is concerned, were found not to be affected by such treatment.

In the second experiment six wild rats were unilaterally adrenalectomized under ether anaesthesia through a ventral mid-line incision which extended from the xiphoid process to the urinary tubercle. After one adrenal was removed it was immediately dissected, weighed and macerated in the usual manner. During this period of time the contents of the abdominal cavity were left spread out exposed to room air. Before the wound was closed, the intestines were handled in a bruising manner between gloved fingers. The anterior abdominal wall was then closed with a continuous suture and the rats returned to their cages. Two hours later they were again anaesthetized and the remaining adrenal was removed for analysis. Six controls were anaesthetized with ether and both adrenals were removed immediately for analysis. It is of interest that the concentration of ascorbic acid in these adrenals was the same as that found in the adrenals of rats which were shot in their natural habitat (Woods, unpublished).

The results from these experiments show that the concentration of ascorbic acid in the two adrenals of a wild rat is approximately the same regardless of whether both glands are removed immediately following induction of ether anaesthesia, or one is removed immediately and the other removed 2 hr later after a second induction. Further, traumatizing the intestines at the initial procedure did not change the results.

Electrical shocks and fighting. The adrenals of sixteen wild rats and of eight domesticated rats were studied after the rats had been subjected to 30 min bouts in the 'fighting chamber' designed by Richter (1950). The individual pairs of rats were placed in the chamber ($9 \times 9 \times 16$ in.) which has a metal grid for a floor. Alternate bars of the grid were connected to the terminals of the secondary coil of a Harvard inductorium so that mild electric shocks could be delivered at will to the feet of the rats. The shocks were tested on the experimenter before the experiment and the secondary of the inductorium was set to produce a shock which was definitely unpleasant to the moistened fingers but below the threshold for muscular contraction.

When wild rats are so stimulated they fight each other. Domesticated rats from our colony rarely fight under such conditions although they do protect themselves from assault if paired with a wild rat in the chamber. In these experiments each pair consisted of either two wild rats or one wild and one domesticated rat. Each pair of rats was kept in the chamber 30 min, during which time they were shocked every 5 sec. At the end of the period of stimulation the rats were returned to their living cages and kept for periods up to 2·5 hr. They were then killed in the usual manner and their adrenals removed for analysis.

Results from ascorbic acid determinations and from examination of Sudan IV preparations in these experiments are similar to the results in experiments using thermal and surgical stress. The domesticated rats showed evidence of marked adrenal activation in the fighting chamber, but no detectable changes occurred in the adrenals of the wild rats.

Adrenocorticotrophic hormone. The effects of ACTH upon adrenal ascorbic acid was determined in twenty-two wild rats. Potency of the hormone preparation (Armour's Lot J—potency stated to be 1 u./mg) was checked in sixteen domesticated rats. Since it had previously been determined that ether anaesthesia did not affect adrenal ascorbic acid in wild rats, these animals were lightly anaesthetized before injection of ACTH. This greatly facilitated the procedure since these animals are difficult and dangerous to handle in the normal state. The domesticated rats were not anaesthetized. ACTH was administered by intramuscular injection and 2 hr later the animals were killed in the usual manner. The results of ascorbic acid determinations are shown in Table 2. It is evident that, when given in sufficient amount, exogenous ACTH is capable of lowering the concentration of ascorbic acid in the adrenals of wild rats. Although more animals must be studied before a dose-response relationship can be determined, the results certainly suggest that the dose of ACTH necessary to elicit a detectable change in the wild rat is considerably higher than the threshold dose for domesticated rats. This lot of ACTH gave a significant result in our colony of domesticated rats when administered intramuscularly in the amount of 10 u./kg body weight.

TABLE 2. The effect of intramuscular ACTH on the concentration of ascorbic acid in the adrenals of wild rats

No. of rats	Dose of ACTH (u./kg)	Adrenal ascorbic acid (μg/mg)	*P*
7	0	$3{\cdot}78 \pm 0{\cdot}16$	—
3	20	$3{\cdot}27 \pm 0{\cdot}39$	$<0{\cdot}1$
8	40	$2{\cdot}39 \pm 0{\cdot}11$	$<0{\cdot}001$
3	60	$2{\cdot}64 \pm 0{\cdot}28$	$<0{\cdot}01$
4	70	$3{\cdot}15 \pm 0{\cdot}19$	$<0{\cdot}05$

TABLE 3. The effect of continuous intravenous ACTH upon the concentration of ascorbic acid in the remaining adrenal following unilateral adrenalectomy in wild rats

No. of rats	ACTH (mg in 3·0 ml. 0·9% NaCl)	Ascorbic acid (μg/mg)	
		Left adrenal	Right adrenal
4	0	$3{\cdot}76 \pm 0{\cdot}16$	$3{\cdot}65 \pm 0{\cdot}24$
4	7·5	$3{\cdot}64 \pm 0{\cdot}21$	$2{\cdot}06 \pm 0{\cdot}17$

A few experiments were conducted in which ACTH was administered by continuous intravenous infusion. The results of ascorbic acid analyses (Table 3) indicate that far less hormone is required when this route of administration is used instead of the intramuscular route. This is in keeping with the findings of other investigators working with other species (Ingle, 1950; Gordon, Kelsey & Meyer, 1951; Wilbur & Rich, 1953). Histological examination of the adrenals of wild rats which had been injected intravenously with ACTH continuously for 3 hr shows that such glands contain far less sudanophilic material than glands from control animals which had been infused with saline.

DISCUSSION

The finding that various forms of stress fail to bring about adrenal cortical metabolic depletion in wild rats but that ACTH does result in such depletion is open to at least two obvious interpretations. First, the stresses used may have been subthreshold for eliciting additional ACTH secretion in wild rats. Another possibility is that the comparatively large adrenal cortex of the wild rat may be able to 'keep up' with endogenous production of ACTH. We are not at present in a position to choose between such alternatives. However, consideration of these results in the light of other available evidence allows for some speculation which may be meaningful.

The effects of acute stress in non-domesticated mammalian forms

The alarm reaction as defined by Selye following his extensive observations upon domesticated animals includes an initial period of adrenal cortical depletion. Sayers & Sayers (1949) outlined several types of stress also as a result of studies carried out exclusively on domesticated rats and guinea-pigs. Sayers's type 1 stress was a sudden, temporary period of 'damage' in which the adrenal cortex was initially depleted of ascorbic acid and lipid with subsequent recovery to control concentrations of these substances. These concepts appear to have gained wide acceptance in recent years.

Several years ago Giroud & Santa (1939) performed experiments upon cats under conditions which were ideal for eliciting an alarm reaction. They found no evidence of adrenal ascorbic acid depletion. More recently, Zarrow & Baldini (1952), Josephson *et al.* (1949), Jailer (1949), Jailer & Boas (1950), Alpert (1950), and Kessler & Leathem (1952) have failed to find evidence of either ascorbic acid or lipid depletion following application of various stresses in non-domesticated animals. To these may now be added the failure of the author to find evidence of adrenal cortical depletion following periods of acute, severe stress in the wild strain of the very species which was originally used to demonstrate the phenomenon. Admittedly, it remains to be seen whether or not some degree of adrenal cortical activation occurs in wild rats and other non-domesticated species under conditions of acute stress. However, there seems to be little doubt now that in many feral species measurable depletion of adrenal ascorbic acid and lipids does not occur under such conditions.

Finally, in any discussion of the adrenal cortex and stress, some work of Vogt (1951) which was recently confirmed by the author (Woods, 1954) must be considered. Vogt found that if domesticated rats are repeatedly exposed to a mild form of stress they rapidly adapt to the stimulus so that, after seven or eight times, a subsequent repetition of the stimulus fails to produce any change in adrenal cortical ascorbic acid, although the first application had evoked an almost maximum depletion. Thus it appears that even the domesticated rat, when removed from its highly protected environment, can adjust its homoeostatic mechanisms so that a sudden change in the external environment no longer results in immediate metabolic depletion of the adrenal cortex.

The role of the adrenal cortex in homoeostasis

A great deal has been written about this subject in the past two decades and much attention has been paid to the activity of the pituitary-adrenal system in emergency states. One recent reviewer (Sayers, 1950) stated that the adrenal cortex plays '...a more ubiquitous role than the sympatho-adrenal medullary system...' in the face of emergency situations. As a matter of fact, there is little direct evidence to support the concept embodied in such discussions. If one accepts such indirect measures as metabolic depletion of the adrenal cortex, then so far only the domesticated rat and guinea-pig can be shown to respond to acute stress with increased adrenal cortical activity. Although there are a few reports of changes in steroid hormone concentration in peripheral blood and urine under such conditions of stress, such results are difficult to interpret in terms of adrenal cortical secretion. Information from experiments in which circulating white cells are counted is even more difficult to equate with the secretory activity of the adrenal cortex. Until it can be shown, preferably in mammals other than domesticated laboratory animals, either that steroid concentrations in adrenal venous blood are increased during or following emergency states or that blood ACTH rises during such periods, it is probably unsafe to make general conclusions.

Doubtless the steroid hormones of the adrenal cortex are necessary for many homoeostatic adjustments in mammals. However, it appears that there is a distinct possibility that many mammals possess well-adjusted homoeostatic mechanisms which are 'set' to take care of most, if not all, emergencies without resorting to large fluctuations in adrenal steroid production and certainly without metabolic depletion of the organ. At least this seems to be a possibility in the case of the wild rat, the cat, the mouse, the hamster, and some avian species. Richter (1954) has provided some evidence which has led him to the tentative conclusion that man, in general, is homoeostatically more comparable to the domesticated than to the wild rat. Until more direct measurements are made in several species including man, we shall not be in a position finally to decide whether stress in man produces effects which are similar to those observed in either domesticated or feral species.

Ascorbic acid and lipid assays as a measure of adrenal cortical function

It is not possible to evaluate changes in adrenal ascorbic acid and lipid content in terms of cortical hormone production. However, the work of Sayers and his colleagues leaves little doubt that physiological activation of the adrenal cortex via endogenous production of ACTH or the injection of foreign ACTH is followed by a decrease in the concentration of these substances in the adrenals of laboratory animals.

If it is correct to assume that the adrenal cortex is activated in a normal manner following ACTH injection, then the finding that exogenous ACTH is effective in reducing the ascorbic acid and lipid content of the adrenals of the wild rat certainly indicates that the methods used in these experiments were capable of detecting at least high levels of adrenal cortical activity. The repeated failure to find evidence of activation under conditions of stress does not, of course, rule out the possibility that adrenal cortical activity was increased somewhat. However, if it was increased, then that increase was considerably less than that produced by the intravenous injection of 0·5 mg ACTH/100 g body weight/hr.

The present findings with ACTH are not consistent with the results and conclusions of Zarrow & Baldini (1952) nor with those of Jailer & Boas (1950) who worked with avian species. Neither are they in agreement with the work of Alpert (1950) nor of Kessler & Leathem (1952) who worked with mammalian species. Although fairly large doses of ACTH were used by these workers (Zarrow, 40–50 mg/kg; Jailer & Boas, 25 mg per chicken; Alpert, 2 mg per hamster; Kessler & Leathem, 60 mg/kg), in no case was the intravenous route of administration chosen. As pointed out previously, continuous infusion has been shown repeatedly to be the most effective method for administration of ACTH. Further, as pointed out by Wilbur & Rich (1953), species differences in threshold to ACTH may be quite large.

The failure of some workers to produce metabolic depletion of the adrenal cortex by ACTH injection in some animals does not necessarily invalidate the methods of assay used. In animals in which failures have been so reported it is necessary to reinvestigate the problem with larger amounts of ACTH preferably administered by continuous intravenous infusion.

SUMMARY

1. No metabolic depletion of the adrenal cortex occurred in wild Norway rats under conditions of acute stress which regularly produced depletion of adrenal ascorbic acid and sudanophilic lipid in domesticated rats.

2. The administration of ACTH did result in ascorbic acid and lipid depletion of the adrenal cortex in wild rats, but the results suggest that a larger amount of hormone is necessary to produce the response in wild rats than is required in the domesticated strain.

3. The validity of ascorbic acid and lipid assays as a method of detecting adrenal cortical activity is discussed. It appears that both assays are capable of detecting at least high levels of activity.

4. Consideration of results in the light of available evidence from the literature suggests that in many mammals the alarm reaction does not include an initial phase of adrenal cortical metabolic depletion. This seems to be so in the case of the wild rat, the cat, the mouse, the golden hamster and in some avian species.

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REFERENCES

Alpert, M. (1950). Observations on the histophysiology of the adrenal gland of the golden hamster. *Endocrinology*, **46**, 166–176.

Bessey, O. A. & King, C. G. (1933). The distribution of Vitamin C in plant and animal tissues, and its determination. *J. biol. Chem.* **103**, 687–698.

Covian, M. R. (1949). Role of emotional stress in the survival of adrenalectomized rats given replacement therapy. *J. clin. Endocrin.* **9**, 678.

Donaldson, J. C. (1928). Adrenal gland in wild gray and albino rat: cortico-medullary relationships. *Proc. Soc. exp. Biol., N.Y.*, **25**, 300–301.

Giroud, A. & Santa, M. (1939). L'acide ascorbique dans la medullo-surrénale. Ses variations supposées en fonction de son excitation. *Bull. Soc. Chim. biol., Paris*, **21**, 1312–1317.

Gordon, E. S., Kelsey, C. & Meyer, E. S. (1951). Adrenal stimulation by intravenous ACTH. *Proc. 2nd Clin. ACTH Conf.* **2**, 30–37.

Hatai, S. (1914). On the weight of some of the ductless glands of the Norway and of the albino rat according to sex and variety. *Anat. Rec.* **8**, 511–523.

Ingle, D. J. (1950). Effect of administering adrenocorticotrophic hormone by continuous injection to normal rats. *Amer. J. Physiol.* **166**, 165–170.

Jailer, J. W. (1949). The pituitary-adrenal relationship in the infant rat. *Proc. Soc. exp. Biol., N.Y.*, **72**, 638–639.

Jailer, J. W. & Boas, N. F. (1950). The inability of epinephrine or adrenocorticotrophic hormone to deplete the ascorbic acid content of the chick adrenal. *Endocrinology*, **16**, 314–318.

Josephson, E. S., Taylor, D. J., Greenberg, J. & Nadel, E. M. (1949). The ascorbic acid content of the adrenal glands of chicks infected with *Plasmodium gallinaceum*. *J. Nat. Malaria Soc.* **8**, 132–136.

Kessler, W. B. & Leathem, J. (1952). An evaluation of adrenal response to ACTH and stress in the mouse. *Fed. Proc.* **11**, 362.

King, H. D. & Donaldson, H. H. (1929). Life processes and the size of the body and organs of the gray Norway rat during ten generations in captivity. *Amer. Anat. Memoirs*, No. 14.

Nichols, J. (1948). Quantitative chemical analysis of the adrenal glands of wild Norway rats. *Proc. Soc. exp. Biol., N.Y.*, **69**, 29–31.

Richter, C. P. (1950). Domestication of the Norway rat and its implications for the problem of stress. *Res. Publ. Ass. nerv. ment. Dis.* **29**, 19–47.

Richter, C. P. (1954). The effects of domestication and selection on the behaviour of the Norway rat. *J. nat. Cancer Inst.* **15**, 727–738.

Rogers, P. V. & Richter, C. P. (1948). Anatomical comparison between the adrenal glands of wild Norway, wild Alexandrine and domestic Norway rats. *Endocrinology*, **42**, 46–55.

Sayers, G. (1950). The adrenal cortex and homeostasis. *Physiol. Rev.* **30**, 241–350.

Sayers, G. & Sayers, M. A. (1949). The pituitary-adrenal system. *Ann. N.Y. Acad. Sci.* **50**, 522–539.

Vogt, M. (1951). The effect of emotion and of beta-tetrahydronaphthylamine on the adrenal cortex of the rat. *J. Physiol.* **114**, 465–470.

Watson, C. (1907). A note on the adrenal gland in the rat. *J. Physiol.* **35**, 230–232.

Wilbur, O. M. & Rich, A. R. (1953). A study of the role of adrenocorticotrophic hormone (ACTH) in the pathogenesis of tubular degeneration of the adrenals. *Johns Hopk. Hosp. Bull.* **93**, 321–335.

Woods, J. W. (1954). Some observations on adrenal cortical function in wild and domesticated Norway rats (*Mus norvegicus* and *Mus norvegicus* var. *albinus*). Doctoral Dissertation, The Johns Hopkins University, Baltimore.

Woods, J. W. (1957). The effects of long-term exposure to cold upon adrenal weight and ascorbic acid content in wild and domesticated Norway rats. *J. Physiol.* **135**, 384–389.

Zarrow, M. X. & Baldini, J. T. (1952). Failure of adrenocorticotropin and various stimuli to deplete the ascorbic acid content of the adrenal gland of the quail. *Endocrinology*, **50**, 555–561.